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Studies on Cambodian Species of *Graphidaceae* (*Ostropales*, *Ascomycota*) (I)

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Three species of two genera, *Fissurina batavana* (Zahlbr.) M. Nakan., Kashiw. & K. H. Moon, *Graphis taneina* M. Nakan., Kashiw. & K. H. Moon and *Graphis chlorotica* A. Massal. in the lichen family *Graphidaceae* are newly reported from Cambodia and their distribution ranges are presented. Among them, *Graphis taneina* is new to science. *G. taneina* is distinct from the allied species of the genus in having sorediate thallus, erumpent lirellae without striation, labia covered by a thallus nearly up to the top of exciples, transversely septate colorless spores which are 30–32 × 7–8 μm and in producing 2-methoxypsoromic acid. A new combination, *Fissurina batavana* (Zahlbr.) M. Nakan., Kashiw. & K. H. Moon is proposed. In addition, these are the second worldwide records for *F. batavana* and *Graphis chlorotica*.

Key words: Cambodia, *Fissurina batavana, Graphis chlorotica*, *Graphis taneina,* lichen.

As part of the Joint Research Project on Conservation of Stone at Ta Nei Temple, Siem Reap supported by the National Research Institute for Cultural Properties, Tokyo, the authors made a botanical survey in order to clarify the lichen flora in Cambodia. During the lichenological investigation in Cambodia from 2005 to 2010 more than 150 specimens of the *Graphidaceae* were collected. Through the taxonomic studies, nine species were reported by Nakanishi et al. (2010). This is the second report of the family in Cambodia.

Materials and Methods

The present study is based on ca 150 specimens of the family *Graphidaceae* collected from 2005 to 2010 by Kashiwadani and Moon near the Angkor ruins, Siem Reap in Cambodia. Specimens reported in the present paper are kept in the herbarium of the National Museum of Nature and Science (TNS) unless otherwise cited. Chemical substances were studied by means of thin-layer chromatography (Culberson and Johnson 1982). Sections of

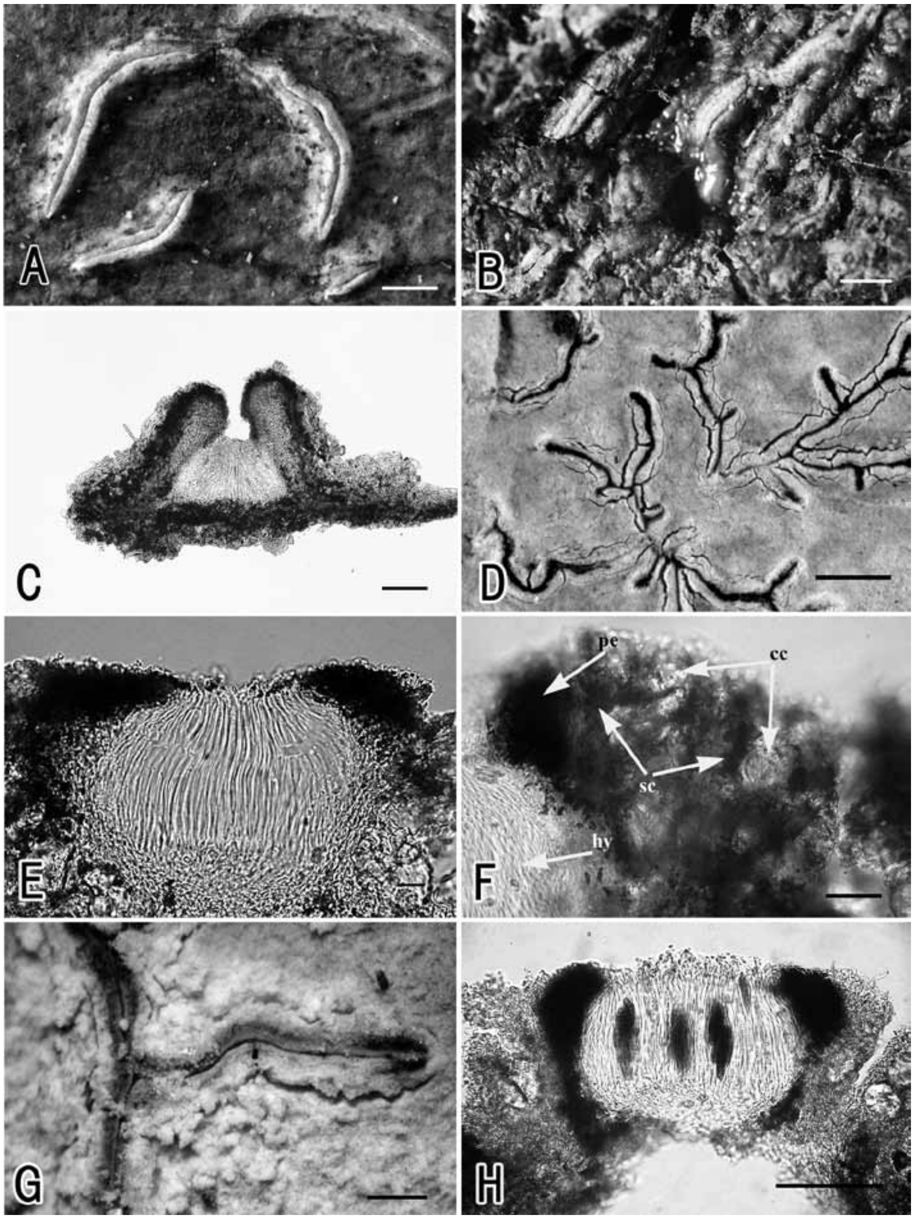

Fig. 1. A–C. *Fissurina batavana* (Zahlbr.) M. Nakan., Kashiw. & K. H. Moon. A. Habit showing lirellae with white pruina [H. Kashiwadani (no. 50184, TNS) & K. H. Moon]. B. Part of holotype (V. Schiffner 2912, W). C. Cross section of an apothecium, showing well-developed and non-carbonized exciples [H. Kashiwadani (no. 50184, TNS) & K. H. Moon]. D–F. *Graphis chlorotica* A. Massal. Moon [H. Kashiwadani (no. 50058. TNS) & K. H. Moon]. D. Habit showing smooth thallus and erumpent lirellae. E. Cross section of apothecia showing apically carbonized exciples. F. Part of labia showing inner striations of thalloid exciple being incorporated with proper exciple (pe), bark cells derived from substrata (sc), hymenium (hy) and calcium oxalate crystals (cc). G, H. *Graphis taneina* M. Nakan., Kashiw. & K. H. Moon [H. Kashiwadani (no. 50118, TNS) & K. H. Moon]. G. Sorediate thallus and lirellae. H. Cross section of an apothecium showing apically carbonized exciples and spores stained by iodine. Scale bars = 1 mm (A, B, D), 25 µm (E, F), 0.2 mm (G) and 100 µm (C, H).

apothecia and thalli were cut by hand-razor and mounted in GAW solution.

Fissurina batavana (Zahlbr.) M. Nakan., Kashiw. & K. H. Moon, comb. nov. [Fig. 1A–C]

Basionym. *Graphis batavana* Zahlbr. in Ann. Mycol. **29**: 76 (1931).

Type collection: Java (Indonesia), in horto botanico buitenzorgensi, ad arborum, V. Schiffner 2912 (W–holotype !).

Thallus greenish tan, glossy, without isidia or soredia, thin, 20–25 µm thick. Apothecia simple to rarely branched; lirellae fissured at the beginning, more or less elevated with age, up to 8 mm long, 0.4 mm wide; labia entire, covered with whitish-gray thalline margin up to the top, striation absent; disc not visible in surface view; exciples cartilaginous, developed laterally, almost absent at the bottom, yellowish brown, uncarbonized, 40–55 µm thick hymenium not inspersed, I –; asci 8-spored; spores colorless, 4-celled (I –), 16–18 × 7–9 µm in size. Secondary substance not detected.

In 1931, Zahlbruckner described *Graphis batavana* Zahlbr. from Indonesia. The holotype specimen (Fig. 1B) was collected on tree bark in a botanical garden in Jakarta. It has well developed apothecia with simple lirellae, uncarbonized exciples (Fig. 1C), 4-locular spores (17–20 × 8–10 µm in size) and produces no chemical substances. Thus, it is considered herewith as a species belonging to the genus *Fissurina*. The Cambodian specimens (Fig. 1A) coincide well with the holotype specimen in morphology and chemistry.

The present species very much resembles *F. rufula* (Mont.) Staiger, a species reported from tropical and subtropical areas in the world (Montagne 1851, Nakanishi 1981, Vainio 1921), which differs in having non-pruinose labia with more or less distinct striations.

Since Zahlbruckner (1931) described this species from Indonesia, no additional record has been made for the species in the world. In the surveys conducted by the authors, this species was found at two localities around Ta Nei temple, where it grows on bark of *Careya* sp. and *Ficus* sp. This is the second report for the species.

Specimens examined: Cambodia. Prov. Siem Reap: Around Ta Nei temple, Angkor ruins, Siem Reap (13°27′N, 103°53′E), on bark of *Careya* sp. tree, elevation about 30 m, July 27 2009, H. Kashiwadani (no. 50116, TNS) & K. H. Moon; the same locality, on bark of *Ficus* sp., H. Kashiwadani (no. 50184, TNS) & K. H. Moon.

Graphis chlorotica A. Massal. in Krempelhuber, Verh. Zool.-Bot. Ges. Wien **21**: 865 (1871). [Fig. 1D–F]

Since Massalongo (in Krempelhuber 1871) described *Graphis chlorotica* based on a collection from Amboina Island in Indonesia (Type collection: Indonesia, Amboina island, on bark, April 1857, coll. ign. s.n., W !, holotype, *tlc* nil acid), no additional collection has been reported from Indonesia or elsewhere, though it was included in the world-wide key for the genus *Graphis* by Lücking et al. (2009).

During the present survey in Cambodia, however, a well developed colony of this species was found on tree bark that coincides well with the type specimen morphologically and chemically. The description based on the Cambodian specimen is as follows: Thallus olive-green to gray-white, smooth, continuous, without isidia or soredia; lirellae erumpent, simple or weakly branched, covered with a lateral thalline margin nearly up to the top, striation absent to very weakly developed; labia dark to pale brown, epruinose; disc not visible from surface view or slightly open in part; proper exciple carbonized apically (Fig. 1E), 100–150 µm thick at the apices, 20–25 µm thick at sides; hymenium not-inspersed, I −; asci 8-spored, spores colorless, with 6–8 transverse septa, 25–37 × 7–8 µm in size, I + purple blue. Chemical substance absent.

Graphis chlorotica is unique in having weakly carbonized exciples only near the apices of labia which often form inner striations being incorporated with bark cells of substrata, hyphae of thallus and calcium oxalate crystals (Fig. 1F).

This species resembles *G. glaucescens* Fée, a species widely disturbed in tropical regions, in having similar exciples carbonized apically, transversely septate spores of a similar size and in producing no chemical substance. However, it can be distinguished from the latter by the continuous thallus and by the absence of white pruina on labia; *G. glaucescens* has thin and dispatched thallus around lirellae which has distinct white pruina on labia and discs.

This species is rather rare in the present area, and it was found only at one locality. This is the second collection for this species in the world, though it was included in the world key of the genus *Graphis* by Lücking et al. (2009).

Specimen examined: Cambodia. Prov. Siem Reap: Ta Nei temple, Angkor ruins, Siem Reap (13°27′N, 103°53′E), on bark, elevation about 30 m. July 19, 2009, H. Kashiwadani (no. 50058, TNS) & K. H. Moon.

Graphis taneina M. Nakan., Kashiw. & K. H. Moon, sp. nov. [Fig. 1G–H]

Similis *Graphi bulacani* sed thallo sorediο hemigloboso, labis estriato et acidium 2-methoxypsoromicum continentibus differt.

Thallus corticolous, pale greenish gray to whitish gray, thin, ecorticate, partly sorediate. Soralia farinose, hemispherical or irregular in shape, 40–100 μm in diameter. Apothecia simple or sparingly branched, up to 4 mm long, 0.18–0.3 mm wide; lirellae erumpent, covered with thalline margin nearly up to the top; labia entire to occasionally striate, covered with white pruina; exciples carbonized apically, 25–40 μm thick near the top, 10–12 μm thick at side; hymenium not inspersed, I−, 90–100 μm high; hypothecium 10–15 μm thick. Asci 8-spored; spores colorless (I + purple), 6–8 transversely septate, 30–32 × 7–8 μm in size.

Type collection: Cambodia. Prov. Siem Reap: Around Ta Nei temple, Angkor ruins, Siem Reap (13°27′N, 103°53′E). On tree bark, elevation about 30 m, 20 December 2005, H. Kashiwadani (no. 50118, holotype in TNS) & K. H. Moon.

Chemistry: 2-methoxypsoromic acid.

Graphis taneina is unique in having sorediate thallus, erumpent lirellae with smooth to partly striate labia (Fig. 1F, G), exciples carbonized apically (Fig. 1H), transversely 6–8 septate colorless spores and in producing 2-methoxypsoromic acid as a chemical substance.

Graphis taneina resembles *G. bulacana* Vain., a species described from the Philippines (Type collection: Luzon, Bulacan, E. D. Merrill 705, TUR-V 27882 !) and *G. glaucescens* Fée reported from tropical areas including Cambodia (Nakanishi et al. 2010), in having similar apothecia with apically carbonized exciples. However, it can be distinguished from the latter by having a sorediate thallus and in producing 2-methoxypsoromic acid; the latter two species have esorediate thalli and produce no chemical substance. It may be possible to be confused with *G. supracola* A. W. Archer, a species reported from Australia and Cambodia, which differs in having exciple carbonized laterally and produces protocetraric acid.

2-methoxypsoromic acid is a rare substance in the family *Graphidaceae*. It was reported from the following species as psoromic acid, which was proved to be 2-methoxypsoromic acid by Elix (Archer 2001); *Fissurina paradoxica* (A. W. Archer) A. W. Archer, *F. psoromica* (A. W. Archer) A. W. Archer and *G. elixii* A. W. Archer (Archer 1998, 2001a, 2001b). However, *Fissurina paradoxica* and *F. psoromica* are distinguished from *G. taneina* in having non-carbonized exciples and muriform spores. *Graphis elixii* differs in having laterally carbonized exciples.

In the present area, this species is rather commonly found on tree barks of *Ficus, Planchonia, Tetramelos,* etc,.

Representative specimens examined: Cambodia. Prov. Siem Reap: Around Ta Nei temple, Angkor ruins, Siem

Reap (13°27′N, 103°53′E), on bark, elevation about 30 m, 19 July 2006, H. Kashiwadani (nos. 49023, 49024) & K. H. Moon.

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文 光喜[a]，中西 稔[b]，二神葉子[c]，柏谷博之[d]：**カンボジア産モジゴケ科地衣類（ビンタケ目，子嚢菌門）の研究 (I)**

筆者等は2005年以来，カンボジア王国のタ・ネイ遺跡周辺で採集した標本に基づいて地衣類の分類学的研究を続けている．2010年には9種のモジゴケ科地衣類を報告した (Nakanishi et al. 2010) が，その後 *Fissurina batavana* (Zahlbr.) M. Nakan., Kashiw. & K. H. Moon，*Graphis chlorotica* A. Massal.，*G. taneina* M. Nakan., Kashiw. & K. H. Moon が新たに確認されたので報告する．*Fissurina batavana* と *Graphis chlorotica* はカンボジア新産種で，世界第二の報告である．また，*G. taneina* は新種として記載された．以下，各種の概略を述べる．

1）*Fissurina batavana*：地衣体は平滑，帯緑褐色でやや光沢がある．リレラは黄土色で単一または僅かに分岐し地衣体から明瞭に盛り上がる．ラビアは灰白色の粉霜で被われ，盤は表面からは見えない．果殻は炭化せず、淡黄褐色．胞子は1子嚢中に8個，無色，4室，16–18 × 7–9 µm．地衣成分は含まない．

Zahlbruckner (1931) は本種を *Graphis batavana* Zahlbr. の名でインドネシア，ジャワ島産の標本をもとに記載したが，その後，どこからも発表された記録はない．タ・ネイ遺跡周辺でも非常に稀で *Careya* や *Ficus* などの樹皮上に生育する．なお，本種の果殻は炭化せず，周糸状体を持つのでスジモジゴケ属として扱うことが妥当と考え，新組み合わせ *Fissurina batavana* (Zahlbr.) M. Nakan., Kashiw. & K. H. Moon を提唱した．

2）*Graphis chlorotica*：地衣体はやや緑色かかった灰白色，粉芽や裂芽はなく連続する．リレラは細く，幅約0.2 mm，地衣体からやや盛り上がり，果托は上部まで地衣体で被われる．ラビアは淡褐色，盤はほとんど見えない．果殻は側面の上部のみ炭化し底部は炭化しない．ラビアと地衣体の間には顕著な溝が見られるが，線状痕は部分的に弱く発生する．胞子は1子嚢中に8個，無色, 6–8個の横隔壁を持つ，25–37 × 7–8 µm．地衣成分を含まない．本種は熱帯に広く分布する *G. glaucescens* に外形や果殻の構造が似ているが，後者は地衣体が不連続で子器の周辺のみに集合し，ラビアが白い粉霜で被われるので区別される．本種はインドネシアのアンボイナ島で記載されて以来，全く記録されていない．調査地内でもきわめて稀で，樹皮上に生じるが生育地は一カ所で確認されただけである．

３）*Graphis taneina*：地衣体は灰白色，連続するが薄い，粉芽を生じる．リレラは細長く（巾0.18–0.2 mm, 長さ1–4 mm）不規則に分枝する．果托は上部まで地衣体で被われるが，粉霜はない．果殻は側面の上部のみ炭化し，底部は炭化しない．子嚢は8個の胞子を生じる．胞子は，無色，6–8個の横隔膜をもつ，30–32 × 7–8 µm無色多室，70–95 × 25–35 µm．化学成分として2-methoxypsoromic acid（P+ 濃黄色，K–）を含む．本種は熱帯に広く分布する上記の *G. chlorotica* に似ているが，地衣体に粉芽を生じ，地衣成分として2-methoxypsoromic acidを含むので容易に区別できる．調査地では普通に見られ，日当たりのよい樹皮上に生育する．

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